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July 4, 1925–October 16, 2000

BY ARNOLD L. DEMAIN AND RALPH F. WOLFE

MARVIN P. BRYANT, EMERITUS professor of microbiology at the University of Illinois, died on October 16, 2000, at his home in Savoy. Illinois, at the age of 75. Marv was born on July 4, 1925, to Melvin Berry and Emna Louise Bucklin Bryant. He was raised on the edge of the foothills in Boise, Idaho, with summer and fall excursions to the family ranch next to the primitive area of the Middlefork of the Salmon River. The environment provided by the area, especially the associations with horses and various ruminants, the freedom and support he received from his parents, and his natural inclination toward biology directed him toward his then unknown goal of doing research in rumen microbiology.

After serving in the U.S. Air Corps during World War II he vowed in 1945 that he would never leave the mountain area, and he completed his diploma at Boise Junior College. In 1946 Marv married Margaret Amelia Betebenna. He started in forestry and switched to soils. Counseling by botany professor Donald Obee moved him into bacteriology and propelled him toward Washington State College in Pullman. According to Marv, his reticence to meet the public and his belief that research and publications alone would

largely satisfy his goals helped to move him toward a career in research.

THE PULLMAN YEARS

In three short years, the general theme of his life's work was set. As a new student with junior standing and with a wife and three-month-old daughter to support, he needed to supplement his GI Bill funds with part-time labor. His first official contact was with Professor Robert E. Hungate, the father of rumen microbiology research and anaerobic microbiology. Hungate was a scientific descendent of the Delft school of microbiology (Biejerinck → Kluyver → van Niel) and was the latter's first American Ph.D. student. Marv started working in Hungate's lab as the glassware washer during the daytime and was later switched to lab work, expediting the research of Hungate and of his Ph.D. student, R. H. McBee. This work was a revelation and better than any possible formal course. The poor funding of research outside of agriculture in this early postwar period made it necessary for Hungate and McBee to make essentially all of their glass apparatus by hand from such materials as Pyrex culture tubes, Erlenmeyer flasks, Kjeldahl flasks, glass tubing, and assorted salvage. Among the things "manufactured" were a complete Warburg apparatus, micro-modification of the Newcomer-Haldane constant pressure volumetric gas analyzer, all condensers, and various units required for the determination of lactate and volatile fatty acids when chromatographic and enzymatic methods were just beginning to evolve.

Among Marv's duties were maintenance of a few stock cultures including the important thermophilic cellulolytic species, *Clostridium thermocellum*; determination of fermentation end products and fermentation balances of various anaerobic species; enumeration and isolation of anaerobic

soil cellulolytic bacteria, *Ruminococcus albus*; and the first isolation of the "less actively cellulolytic rod" later named *Butyrivibrio fibrisolvens*. In association with the General Electric Research Laboratory in Schenectady, N.Y., Hungate outlined experiments in which Marv did in vitro rumen fermentations of wood treated with cathode rays. Results showed that the cellulosic fraction of wood was released from lignin and made available to anaerobic microbial digestion and volatile acid production, while the lignin remained indigestible at certain radiation dosages.

In June of 1949 he received his B.S. degree, and Hungate, having acquired funds for research from state liquor tax money, placed Marv on a half-time research assistantship at a salary of about $1,500 a year. His research involved the isolation and characterization of the small rumen spirochete of the genus *Treponema*, which could move through agar or particulate forage and compete with cellulolytic bacteria for use of the soluble sugar energy sources produced by the latter from cellulose. The report of his research was the first published work on fermentation products of a spirochete and suggested strong metabolic interactions between cellulolytic and noncellulolytic fermentative bacteria in anaerobic ecosystems, a finding that only later received extensive documentation. Electron micrographs of the aging cells taken with the aid of a physics graduate student showed the periplasmic membrane and fibrils and protoplasmic cylinder of spirochetes, but these were not recognized until much later.

Because Marv wanted to continue on to his Ph.D. at Pullman and Hungate believed that he needed more interactions with course work and research in another area of the country, Marv continued his assistantship through Washington State College for an eight-month period in 1949 at Cornell University. During this period he took course work

in animal nutrition, bacterial metabolism, industrial microbiology, and bacterial cytology. He also had a large number of associations with other professors and students in both veterinary medicine and bacteriology. These included H. H. Dukes, professor of veterinary physiology; James M. Sherman, professor of bacteriology and long-time editor of the *Journal of Bacteriology;* and Meyer J. ("Mike") Wolin, then an undergraduate student. Marv's research was conducted in association with Professor Robert Dougherty on the microbiology and physiology of acute indigestion, lacticacidosis, in sheep. *Streptococcus bovis* was found to be the initiator of high rumen lactate and was often followed by members of the genus *Lactobacillus*.

During his stay in Ithaca, Professor William Pounden of Ohio State University visited and carried word to Beltsville Agricultural Research Center that Hungate had a graduate student working in rumen microbiology. A research bacteriology position was offered by the Bureau of Dairy Industry and, after a strong push from Hungate, Marv decided to accept.

THE BELTSVILLE YEARS

Having received his M.S. degree in 1950 from Washington State College, Marv was concerned about completing his Ph.D. degree and this was made possible at his own expense and time by Lane A. Moore, head of the dairy cattle nutrition group at Beltsville, and Professor Raymond Doetsch, Department of Microbiology, University of Maryland, College Park, just a short distance from Beltsville. He was allowed to take one course per semester at College Park and his thesis research was completed while still working at Beltsville. He received his Ph.D. degree from the University of Maryland in 1955.

Marv's faith in his ability to adequately advance basic

knowledge on rumen microbiology was very low, and during the first few years at Beltsville he very much missed Hungate's guidance. He was given essentially complete freedom to work within the huge area of rumen microbiology by Moore, who was an outstanding nutrition expert and research administrator, but who could offer essentially no guidance in microbiology. Marv's main goals were to taxonomically describe, with emphasis on ecologically important metabolic features, the numerous species of rumen bacteria and to chemically identify "unusual" growth factors present in rumen fluid but not in most organically rich growth media. Hungate had worked mainly with pure cultures of the cellulolytic population and his development of classic anaerobic techniques and habitat-stimulating growth media had largely removed the blocks confronting further advances in isolation and description of rumen bacteria. However, Hungate was already beginning to feel that kinetic studies of major overall rumen biochemical reactions were more important to his research effort. In addition, Doetsch was formulating the idea that the "physiological" approach using washed suspensions of mixed rumen microorganisms would probably yield the most fruitful results in the future. There were thus major currents against Marv's emphasis on pure culture studies, but he continued this emphasis with apprehension.

During Marv's early research, the general field of anaerobic bacteriology remained in a chaotic state because of a general lack of knowledge about anaerobic bacteria, the failure of most workers to determine catabolic products of energy metabolism, and the inability of most microbiologists to grow and isolate the relevant species. Techniques such as determination of taxonomically important cellular constituents, percent guanine plus cytosine in the DNA, and DNA-DNA hybridization were for the most part yet to

be developed. Even with the development of these methods and various others, many problems of relatedness among major microbial taxonomic groups still existed. When his work at Beltsville was initiated, the only rumen anaerobes that had been studied and named with enough detail for other workers to identify were the cellulolytic species, *Bacteroides succinogenes* by Hungate and *Ruminococcus flavefaciens* by Sijpesteijn. Hungate had published on various *Ruminococcus* species and *B. succinogenes* earlier but had not named them, and Sijpesteijn's work at Delft had been delayed for several years by the German invasion of Holland. Marv's first concern was to develop and evaluate methods for enumerating and isolating the more numerous bacterial species. The anaerobic roll-tube methods of Hungate were somewhat modified for larger scale studies. The 40 percent rumen fluid-glucose-cellulose agar roll tube (RGCA) medium with CO_2 gas phase, bicarbonate as the main buffer, and cysteine as added reducing agent was a slight modification of Hungate's selective cellulose agar medium and allowed the isolation of most of the predominant carbohydrate fermenting species. Traces of O_2 were removed from the commercial gases that were passed through a glass column containing hot copper filings. This column turned dark as the copper oxidized and could be quickly reduced by exposure to H_2. A balanced mineral anaerobic solution similar to the growth medium, but with rumen fluid and sugar energy sources removed, served as the rumen fluid diluent. Large numbers of bacterial strains were rapidly isolated by picking colonies from large-diameter (18 mm) roll tubes with platinum-iridium inoculating needles and stabbing them into deep slants containing a reduced amount of agar. The main concentration of cells in the water of syneresis and in the top part of the agar at the base of the slant allowed even small colonies of the more

fastidious anaerobes to grow. It also provided an excellent menstrum for preparing wet mounts for study of cell morphology and motility with the phase-contrast microscope. Marv used various modifications for studying anaerobic bacteria as diverse as methanogens, photosynthetic bacteria, sulfate reducers, human GI tract anaerobes, and those of medical concern.

A large number of bacterial strains were first isolated and enumerated from dairy cattle fed diverse diets, such as hay, hay-concentrate, and forage-crop silage. These strains were studied for morphology and a few physiological features and were placed in tentative groups. Selected strains were then placed in a dry-ice cabinet so that live cultures could be maintained for detailed biochemical and nutritional studies. Unlike Hungate, Marv firmly believed that one of his most important functions was maintaining the culture collection of rumen anaerobes, many of which were later well documented as important in anaerobic degradation, and making them available to other researchers around the world. With the continuity of support at Beltsville and later at the University of Illinois he was able to continue this function. For about 20 years his group was the main and often the only source of many rumen anaerobes.

Among the important bacteria described in detail were many cellulolytic strains of *B. succinogenes*, which until detailed studies were done seemed morphologically quite different from Hungate's strains. Marv later showed that this species degraded highly resistant crystalline cellulose much faster than other known mesophilic anaerobic cellulolytic bacteria. Large numbers of cellulolytic strains of *Ruminococcus albus* and *R. flavefaciens* were studied and the work also emphasized the importance of these in xylan fermentation and the importance of ethanol formation in *R. albus* versus succinate formation in *R. flavefaciens*. The

genus *Butyrivibrio* was named and great versatility in production of CO_2, lactate, and butyrate and in fermentation of important carbohydrates was found, as well as considerable variation between strains. Only a few strains fermented cellulose, but most fermented xylan, starch, pectin, and many other carbohydrates. Later work at Illinois in association with Jones, Cheng, and Simpson from Canada showed that some strains fermented important plant flavonoids. This work represented the first pure culture demonstration of anaerobic degradation of the aromatic heterocyclic ring structure.

Bacteroides ruminicola, one of the most numerous and versatile of rumen bacteria, was isolated and described. This succinate and acetate-producing species fermented complex carbohydrates including various pentosans, pectin, and starch, as well as many sugars. It was actively proteolytic and could convert amino acids, derived from peptides transported into the cell, into ammonia, CO_2, and various straight- and branched-chain volatile fatty acids.

Selenomonas ruminantium had been observed microscopically in rumen contents as early as 1889 because of its unique morphology and relatively large size and was obtained in pure culture but not recognized by Huhtanen and Gall in 1953. Marv isolated and identified this important rumen species, which produced various amounts of propionate, acetate, lactate, and CO_2 from many different sugars and starch and also degraded a number of amino acids. The subspecies *lactilytica* fermented important energy sources such as lactic acid and glycerol. *S. ruminantium* became one of the organisms most used by workers at Illinois and elsewhere in rumen microbiological studies. For example, Marv used it to examine various interactions between species, the effect of factors such as growth rate on the types of fermentation products, the various enzyme sys-

tems involved in ammonia assimilation, and regulation of urease production. A number of additional new species and often new genera involved in various rumen reactions were also described. These included the butyrate-forming *Eubacterium cellulosolvens*, pectin-fermenting *Lachnospira multiparus*, xylan-fermenting *Eubacterium ruminantium*, and the succinate- and acetate-forming *Succinivibrio dextrinosolvens* and *Succinimonas amylolytica*.

Rumen flora developing in young calves were studied concurrently with the detailed investigations of bacteria from mature animals. The studies of the flora of young calves gave Marv further insight into the diversity of anaerobic bacteria and the difficulty of identifying well-studied strains from previously published descriptions, which were still very poor in 1958. Most of the large number of anaerobes in calves one to three weeks old differed substantially from those in mature animals and Marv decided not to name them. However, it became possible to identify most representative strains, partly because of the efforts of W. E. C. Moore and his colleagues at the Anaerobe Laboratory of Virginia Polytechnic Institute. One of the most important strains that Marv identified in young calves was *Fusobacterium necrophorum*. Although long known as a pathogen and major cause of liver abscesses in cattle, it was not known as a major normal organism fermenting lactate, amino acids, and sugars and producing butyrate and other acids in young calves. Another important strain was *Clostridium clostridiiforme*, an organism whose spores were very hard to detect. It had been found in several pathologic processes and as a normal poultry intestinal isolate but had not been previously found in the rumen.

Marv's group, as well as workers in Scotland, discovered the long form of *Lactobacillus vitulinus* in young calves. This organism obviously differed from the short form found

in older calves and adult ruminants. Both Marv and the Scottish workers also described the lactate-fermenting, amino-acid-catabolizing *Megasphaera elsdenii* that had been studied in detail by Elsden and Lewis in 1953. In addition, the lactate-fermenting *Eubacterium limosum* (*Butyribacterium rettgeri*) was detected in the rumen for the first time. This organism was of interest because of its ability to produce butyrate and longer-chain volatile fatty acids from one-carbon compounds such as methanol or H_2-CO_2.

The studies of pure cultures of functional rumen bacteria disclosed large numbers of species that produced such products as lactate and ethanol, which were not normal products or important extracellular intermediates in the rumen fermentation. Some microbiologists believed, because these "artifacts" occurred, that the pure cultures were not worth studying. However, Marv's view was that judicious studies of pure cultures and known mixtures were valuable because they would yield important facts about the environmental factors causing the abnormalities that would be impossible to find with studies on the total fermentation. These facts in turn would lead toward better knowledge about regulation of the rumen fermentation. Later work at Illinois and elsewhere strongly supported Marv's view.

After initiating the studies on numbers and kinds of rumen bacteria, Marv was joined by Nola Small, an outstanding technical assistant. As a result, he had time to do detailed studies on the nutrition of pure cultures. Hungate's studies had shown that a number of rumen cellulolytic species required unknown growth factors that were present in rumen fluid but not in rich sources of vitamins and other growth factors such as liver or yeast extract. Marv found that the factor required by *B. succinogenes* had two components. A straight-chain saturated fatty acid, *n*-valerate, or longer-chain acids satisfied one component, and a branched-

chain acid such as D-2-methyl-*n*-butyrate or iso-butyrate satisfied the other one. This was the first indication that some anaerobic bacteria required acids produced by other rumen microbes from certain amino acids and, in the case of *n*-valerate, also from carbohydrates. Finding these factors allowed Marv to establish a chemically defined minimal medium for *B. succinogenes*. This was the first formulation of such a medium for an important rumen anaerobic bacterium. Another first was a discovery that had never before been made for a nonmarine bacterium: *B. succinogenes* required a large amount of sodium ion for growth. Later studies by Don Caldwell at the University of Wyoming showed that many other rumen anaerobes were moderate halophiles (i.e., they required moderate amounts of sodium ion for growth). Thus, the rumen had some features of an inland sea.

Marv's first professional colleague, Milton Allison, joined him in 1957 and was put to work on the unknown nutrient requirements of the cellulolytic genus *Ruminococcus*. They found that many ruminococci required one or more of the branched-chain fatty acids isovalerate, isobutyrate, and 2-methyl-*n*-butyrate for growth. Detailed studies of a strain of *R. flavefaciens* using position-labeled ^{14}C-isovalerate established that these acids were needed for (1) certain amino acid biosyntheses via previously unknown reductive carboxylation reactions and (2) biosynthesis of cellular lipids (long, branched-chain fatty acids and aldehydes). These studies were the first of a series from Marv's lab showing that many rumen bacteria, other heterotrophic bacteria, and most methanogenic anaerobic bacteria had a very limited ability to utilize organic nitrogen sources such as amino acids or peptides. Instead, they utilized ammonia as the essential and major nitrogen source and utilized CO_2 and various volatile fatty acids, such as acetate and those indicated above,

as major sources of carbon but not of energy. The concept was advanced that the rumen environment, being quite low in soluble organic nitrogen and being high in ammonia, CO_2, and volatile fatty acids, had evolved and selected many bacterial species that utilize these materials for amino acid and lipid biosynthesis, and that these bacteria had lost or never gained the ability to utilize many preformed amino acids or to biosynthesize all the carbon skeletons of some amino acids. The work with Allison on the ability of *Ruminococcus* to convert ^{14}C volatile fatty acids into lipid was done in association with Professor Mark Keeney and Ira Katz of the University of Maryland. The results showed for the first time that rumen bacteria contain a large amount of *iso* and *anteso* long branched-chain fatty acids, as well as considerable long-chain aldehydes, which were shown to be present in the plasmalogen class of lipids of the cellular phospholipids. This was the first demonstration of plasmalogens in bacteria and led to the concept that many of the branched-chain and odd-numbered carbon fatty acids of ruminant milk and body fat were biosynthesized by bacteria in the rumen. Use of the then rapidly developing methodology for chromatographic separation of lipids and fatty acids was essential in these studies.

Further studies in association with another technical assistant, Isadore M. ("Ike") Robinson, established that most functional rumen bacteria could be grown in relatively simple chemically defined culture media. Many species used ammonia, rather than free amino acids or peptides, as the major and essential source of nitrogen, and all species were able to utilize ammonia as their main nitrogen source. However, a very significant number preferred a complex mixture of exogenous free amino acids. One major species, *B. ruminicola*, was found to require heme, which was shown to be biosynthesized by many other rumen bacteria. While

B. ruminicola strongly preferred ammonia to free amino acid nitrogen and carbon, it took up little ammonia when peptides were available as nitrogen sources. Further studies with Ken Pittman showed that this species transported and utilized oligopeptides very effectively as nitrogen and carbon sources for growth. However, it was ineffective in utilizing free amino acids or dipeptides as compared to ammonia or the preferred oligopeptides. These results emphasized the importance of peptides in the nitrogen economy of the rumen. Some strains of diverse bacterial species had proteolytic ability. Marv's work with Howard Bladen showed that among predominant bacteria from mature animals, only a few species, such as *S. ruminantium*, *M. elsdenii*, and *B. ruminicola*, could catabolize amino acids or peptides to ammonia and volatile fatty acids.

Marv's nutritional studies laid the foundation for development with D. R. Caldwell of a growth medium in which rumen fluid could be replaced by better standardized ingredients for enumeration and study of most rumen bacteria and of many bacteria from other anaerobic microbial ecosystems. With Caldwell, studies were initiated to determine the specificity of the heme requirement of *B. ruminicola*. David White of the Rockefeller Institute was an expert in using difference spectra and other techniques for analyses of cytochromes of aerobic bacteria. Taking Marv's anaerobic culture media and other culture paraphernalia to Rockefeller, Marv and David began a search for cytochromes as the possible reason for the heme requirement of *B. ruminicola*, though cytochromes were then not known to be functional in fermentative anaerobic organisms. They found that *B. ruminicola* contained a b-type cytochrome involved in electron transport for fumarate reduction to succinate by reduced pyridine nucleotide generated in glycolysis during the CO_2-dependent fermentation of carbohy-

drate. This was the first experimental evidence suggesting that cytochrome-linked electron transport was involved in energy transformations needed for growth of strictly anaerobic fermentative bacteria.

Because of the difficulty of isolating methane-producing bacteria, these organisms had received little attention until the 1950s, when Hungate emphasized the importance of their ability to utilize the H_2 produced by fermentative bacteria. The H_2 reduced CO_2 to form methane as the source of energy for the organisms' growth. Hungate and P. H. Smith isolated and described the major H_2-using methanogen of the rumen *Methanobrevibacter ruminantium* in 1958, and indicated that it needed unknown factors in rumen fluid for growth. At that time, they were not interested in further studies on the nutrition of this species. Thus, Ike Robinson and Marv, being interested in the nutrition of rumen organisms, isolated the methanogen using the techniques previously developed but with hydrogen gas replacing sugars as the energy sources. They confirmed the numerical importance of *M. ruminantium* and its need for unknown growth factors in rumen fluid. The growth assays used to determine nutrient requirements were very tedious, and studies were also complicated by the number of different "rumen fluid" factors required. They found three different rumen fluid factors. One was 2-methyl-*n*-butyrate. The second was acetate, subsequently shown by AI Joyner, a postdoctoral student all Illinois, to be the exogenous source of 60 to 70 percent of the cell carbon in this bacterium. This held even when the growth medium contained large amounts of preformed cell monomers such as amino acids and peptides, which heterotrophic bacteria usually prefer as carbon and nitrogen sources. This discovery, together with Marv's demonstration that ammonia was essential as the main source of nitrogen, showed that this H_2-CO_2-using

chemolithotrophic organism resembled the major cellulolytic and some other rumen fermentative bacteria in certain major nutritional features. Because of his work at Illinois and later efforts of several other laboratories, it is now known that all species of methanogens so far studied require ammonia as the main nitrogen source and that acetate is often a preferred major source of cell carbon. The third rumen fluid factor required by *M. ruminantium* was shown by Robinson and Marv to be a quite strong acid that was much more polar than the two fatty acid factors. It could not be separated from rumen fluid by acid ether extraction, but could be separated from the residue of ether-extracted rumen fluid into two components by anion exchange resin chromatography. It was a highly stable organic compound with a low molecular weight and was mainly but loosely associated with rumen microbial cells rather that the fluids. They could not detect it in many other crude or defined materials they used to grow nutritionally exacting bacteria.

In 1963 Marv was invited to the Third Rudolfs Conference at Rutgers University to give a paper on "Bacteriology of the Rumen" under the theme "Principles and Applications in Aquatic Microbiology." At Beltsville he was somewhat isolated from researchers involved in major areas other than rumen microbiology, and this conference provided the setting for him to meet a large number of scientists who had distinguished careers in related areas. Marv later identified Perry McCarty, a sanitary engineer at MIT and Stanford University working on kinetics and physiological factors in sewage sludge methanogensis, and Ralph Wolfe, Department of Microbiology, University of Illinois, as two that greatly influenced the direction of his future research. Early in 1964 he was invited to give guest lectures in Wolfe's course, Microbiology 309, and in the Department of Dairy Science seminar, and negotiations began concerning a pos-

sible move to the University of Illinois. The prospect of being closely associated with good basic microbiological research such as that in the laboratories of Meyer ("Mike") J. Wolin and Wolfe, the anticipated interaction with excellent ruminant nutritionists such as Dick Brown and Carl Davis, and the interest and encouragement of Glenn Salisbury, then head of the department, M. B. Russell, director of the Experiment Station, and other department members, such as Harry Broquist, were too much for Marv to resist. In addition, Urbana was 800 miles closer to Idaho. His tenure at Beltsville had been very pleasant and he had exceedingly good relationships with many excellent ruminant nutritionists including Lane Moore, Bill Flatt, Peter van Soest, and Dale Waldo. However, the lack of interaction with more basic areas of microbiology and the inability to obtain research grants from other government agencies (due to USDA restrictions) or funds for a number of needed but expensive scientific instruments were among the reasons that Marv gave for leaving Beltsville after 13 years.

UNIVERSITY OF ILLINOIS

In 1964 Marv moved with his family to Illinois to be professor of microbiology in the Department of Dairy Science and Microbiology. He brought in the culture collections, continued work on the nutrition of methanogens, and initiated various other projects. His expertise in growing methanogens on hydrogen gas and CO_2 turned out to be crucial to many of the advances made in the Dairy Science Microbiology Division labs in collaboration with Wolin and in the laboratory of Wolfe in the Department of Microbiology.

After his move to Illinois, work on the unknown factor required by *M. ruminantium* was continued for several years with research associate Olga Nalbandov and technician Ken-

neth Holmer. They finally obtained enough relatively pure factor from 5 liters of rumen fluid to grow more than 20 liters of the methanogen using various extractions, absorption, and column chromatographic methods. However, the dry weight of the factor was too small and they failed to get enough material to determine chemical structure. Through the late 1960s and into the 1970s Wolfe's graduate students Barry McBride and later Craig Taylor were working on isolation and chemical characterization of a new coenzyme that was found in most methanogens and was required for methyl transfer reactions in the terminal stage of intracellular methane formation. The coenzyme seemed to be identical with the highly polar growth factor that was required by *M. ruminantium* and that Marv had isolated from rumen contents though not in large enough amounts for chemical characterization. In early 1974 McBride and Taylor characterized coenzyme M as 2-mercaptoethanesulfonic acid, chemically synthesized it, and gave Marv some to test as the growth factor required by *M. ruminantium*. The tests showed conclusively that the coenzyme was indeed the elusive growth factor. Only 3 to 5 nanograms of the coenzyme per ml of medium were required for half-maximal growth.

During the same period Marv's graduate student Sin-Fu Tseng worked out another enzymatic assay of *M. ruminantium*. His purpose was to determine the coenzyme(s) involved in electron transport during H_2 oxidation and the pyridine nucleotide reduction involved in the organism's energy metabolism and methane production. An unknown factor, apparently different from ferredoxin (often functional in anaerobes), was found to be involved. Previous workers in Wolfe's laboratory had isolated a chemically unknown compound with a relatively low molecular weight and with blue-green fluorescence in ultraviolet light and had found large amounts in other methanogens. In cell

extracts of various methanogens in the presence of hydrogen the compound lost fluorescence and strong light absorption at 420 nm. The factor was named F (factor) 420 and F_{420} isolated from *Methanobacterium bryantii* was made available to Marv and Tseng. They found that F_{420} was the coenzyme necessary for transfer of electrons generated in H_2-oxidation to pyridine nucleotides in both *M. ruminantium* and *M. bryantii* and also in formate oxidation to CO_2 in the former. It thus became evident that methanogens contain a number of coenzymes different from those in other life forms.

Methanobacillus omelianskii was the methanogen discovered by H. A. Barker in the 1930s and isolated from San Francisco Bay mud in the 1940s. Its energy metabolism involved oxidation of ethanol to acetate with the electrons generated being used to reduce CO_2 to methane. Nongrowing suspensions of cells that had been grown on ethanol-CO_2 were known to use H_2 to reduce CO_2 to methane. Because of its ability to grow on the soluble substrate ethanol, *M. omelianskii* was easier than other methanogens to grow with techniques then in vogue. As a result of biochemically oriented studies in Barker's lab, and especially a collaborative effort starting in about 1960 between Wolfe and the Wolins at Illinois, more became known about the biochemistry of methane formation in *M. omelianskii* than in any other methanogen. When Marv arrived at Illinois, Wolfe had expressed some doubts about the purity of the culture because slightly differing cell shapes were sometimes seen in cultures and he felt that these might represent two species. He suggested that Marv isolate colonies from the ethanol-grown culture by growing them in H_2-CO_2 agar roll tubes using techniques previously used for *M. ruminantium*. To Marv's surprise, colonies grown in large numbers from H_2-CO_2-grown cells and liquid cultures grown in the same man-

ner failed to grow or utilize ethanol when returned to the ethanol medium without hydrogen. The hydrogen-utilizing colonies of methanogens utilized only H_2-CO_2 as energy source. Later work in Wolfe's lab and elsewhere showed that these colonies constituted a new species of methanogen and were present in several ecosystems.

Using ethanol-CO_2 roll tubes without H_2 gas, Marv then isolated a nonmethanogenic ethanol-utilizing species, called "S-organism," from the ethanol-CO_2 culture of *M. omelianskii*. The S-organism produced only tiny colonies and little growth in pure culture and utilized only a very small amount of ethanol while producing acetate and H_2. Study of changes in free energy of the reaction indicated that in pure culture, the accumulation of a very small amount of H_2 would stop the ethanol-utilizing reaction needed for growth. The effective ethanol fermentation could be carried out by recombining the two species isolated *from M. omelianskii* or by combining the ethanol-utilizing S-organism with any H_2-using methanogen. This was the first demonstration of a syntrophic association of two microbial species involving "interspecies H_2 transfer" (a term coined by Wolin). The phenomenon is now known, mainly from the work at Illinois, to be of great importance in anaerobic degradation in the rumen. It is considered even more important in methanogenic ecosystems where more complete anaerobic degradation occurs (e.g., where products such as volatile and longer-chain fatty acids are largely converted to methane and $CO_{2)}$.

C. A. Reddy, having completed his M.S. work showing that several anaerobes of the human gastrointestinal tract and other anaerobes from the rumen contained cytochromes involved in fumarate reduction to succinate, was given the Ph.D. problem of further characterizing the S-organism and the enzymology involved in its production of acetate and H_2 from ethanol. The work showed that the organism had a

normal type of ethanol dehydrogenase linked to reduction of pyridine nucleotide and that the reduced pyridine nucleotide was reoxidized by H_2 production via a ferredoxin-linked hydrogenase. The acetaldehyde formed was oxidized to acetate via ferredoxin-linked aldehyde dehydrogenase and hydrogenase. The microbial production of H_2 from low redox potential electrons generated from aldehyde or 2-ketocarboxylic acids such as pyruvate via dehydrogenases linked to ferredoxin-linked hydrogenases had been known for some time. However, the production of H_2 from higher redox potential electrons (reduced pyridine nucleotide) generated from alcohol oxidation or glycolysis was generally believed to be impossible. The work of Reddy and Marv documented that the production of H_2 via these latter reactions was in fact energetically very favorable when H_2 concentration was kept low by means of H_2-using organisms such as methanogens.

Norbert Pfennig from Göttingen, Germany, worked in Marv's lab briefly and showed that S-organisms could be grown fairly effectively on pyruvate in pure culture. Reddy showed that this fermentation involved production of acetate and ethanol, ethanol being the main electron sink product of pyruvate rather than H_2, which was produced in only small amounts. When the S-organism was combined with a methanogen, pyruvate was more rapidly degraded to acetate, CO_2, and hydrogen. The hydrogen did not accumulate because it was used by the methanogen, and little or no ethanol was produced. When co-cultured with the methanogen, the S-organism grew more efficiently, presumably because little energy becomes available for growth when pyruvate is degraded to ethanol whereas pyruvate fermentation to acetate, CO_2, and H_2 provides this energy. This work provided a probable explanation for the fact that many rumen and other bacteria produced ethanol and/or lactate

in addition to acetate and H_2 in pure culture where H_2 accumulated but did not usually produce ethanol or lactate in the natural ecosystem, where they were closely associated with H_2-using methanogens or other H_2 utilizers. This idea was confirmed by Marv's work in association with Wolin and his students. In model experiments with *R. albus*, the organism produced more acetate and H_2 and no ethanol when co-cultured with an H_2 utilizer on carbohydrate energy sources.

Later work in Wolin's laboratory and elsewhere provided further evidence that reduced pyridine nucleotide produced in glycolysis by fermentative bacteria could be reoxidized via H_2 production if methanogens use the H_2 efficiently. The efficient use of H_2 allowed the fermentative bacteria to produce larger amounts of H_2 and acetate and smaller amounts of ethanol, lactate, succinate, propionate, and other reduced products of pyruvate. Also other ruminant nutritionists and microbiologists were beginning to realize that although only very small amounts of H_2 accumulated in the fluids of the microbial ecosystem, changes in the level could greatly affect the amount of acetate as compared to propionate and other reduced products of the ecosystem, and therefore influenced the ruminant animal's efficiency in producing milk or meat.

Unlike the carbohydrate-fermenting anaerobes where H_2 concentration affects only relative efficiency of growth and kinds of products produced, the S-organism from *M. omelianskii* could not grow on the natural exogenous substrate ethanol unless the H_2 concentration was maintained at a very low level. That is, the only way to dispose of electrons (reduced pyridine nucleotide) generated in oxidation of ethanol to acetate was via pyridine nucleotide-linked H_2 production. The organism was incapable of producing other electron-sink products. The term "obligate proton re-

ducing (H_2-forming) acetogenic bacteria" was coined in association with Rolf Thauer and Wolfe for this previously unknown metabolic type of bacteria. Wolin hypothesized that other species that would grow anaerobically only in the presence of H_2 utilizers would be found.

Of chief interest to Marv were the bacteria involved in the important beta-oxidation of fatty acids containing even-number carbon atoms to acetate and of odd-number carbon fatty acids to acetate and propionate, and the presumably different species that oxidatively decarboxylate propionate to acetate and CO_2. Quite good documentation already existed that the one- and two-carbon fatty acids, formate and acetate respectively, were degraded by pure species of methanogens producing methane and CO_2. It was generally believed that single species of methanogens carried out the various oxidations of the three-carbon fatty acid, propionate, and longer-chain fatty acids, and disposed of electrons generated via CO_2 reduction to methane. Although several species carrying out these reactions had been named, none had been obtained in pure culture. Marv expected to find additional species that carried out these fatty acid oxidations in obligate syntrophy with H_2-using methanogens. He further expected that because of the change in free energy of the probable reaction involved, these species would require much lower concentrations of H_2 in the environment than even the ethanol degraders.

Studies showed that species of the genus *Desulfovibrio*, which in pure culture grew and degraded lactate or ethanol to acetate only when the electrons generated in the oxidation were used to reduce sulfate to sulfide, could be grown in the absence of the electron acceptor but in syntrophic association with H_2-using methanogens. This established a previously unknown ecological niche for sulfate-reducing bacteria. In further studies done by graduate

student Mike McInerney, co-cultures of *Methanosarcina* (an organism that produces methane from both H_2-CO_2 and acetate) and *Desulfovibrio* completely dissimilated lactate or ethanol to methane and CO_2. More important, the degradation of acetate by the *Methanosarcina* was repressed until after the organism had utilized the H_2 that the *Desulfovibrio* produced while dissimilating the primary substrate to acetate. This finding was relevant to understanding the fact that although *Methanosarcina* is present in the rumen, it uses H_2-CO_2 (and probably methanol and methylamines) as energy source in preference to acetate degradation except under adverse conditions of very long rumen retention times and extremely low H_2 levels.

Marv had worked for many years without success to prove via isolation of co-cultures that propionate and longer-chain fatty acids were anaerobically degraded in nature by syntrophic associations of fatty acid oxidizers with H_2 utilizers. Success was finally achieved in 1976, while he was on sabbatical leave with Norbert Pfennig in Germany. The success was due to the use of the fatty acid oxidizers with sulfate-reducing *Desulfovibrio* as the H_2 user in place of *M. ruminantium*, the former apparently having much greater affinity for H_2 than the latter at the slow growth rate necessary for the fatty acid degrader. After the initial success the project was expedited by McInerny. The organism, which was named *Syntrophomonas wolfei*, beta-oxidizes fatty acids producing H_2 and either acetate (from fatty acids with even-numbered carbon atoms) or acetate and propionate (fatty acids with odd-numbered carbon atoms) in obligate syntrophy with H_2-using methanogens such as *M. hungatei, Methanosarcina,* or H_2-using *Desulfovibrio*. This was the first description of pure co-cultures of anaerobic bacteria that degrade fatty acids. Later, postdoctoral associate David Boone isolated a propionate-decarboxylating, acetate-producing

species that he and Marv called *Syntrophobacter wolinii*. This organism required syntrophic conditions similar to those of *S. wolfei*.

Marv's graduate student Vincent Varel found that thermophilic methane production could be started up from bacteria in cattle waste in a period of about 12 days, a much shorter time than previously thought possible. They obtained a faster methanogenesis with higher loading of cattle waste into digestors than any previous group had achieved. The work was continued by research associate Rod Mackie who greatly expanded the knowledge of the kinetics of fatty acid degradation, bacterial growth, and protein synthesis in cattle-waste methanogenesis at both mesophilic and thermophilic temperatures.

Although ruminant nutritionists had long been interested in the amount of microbial cells and protein synthesized in the rumen in relationship to the amount of organic matter the microbes digested, considerable difficulty was involved in accurately determining this in vivo, and yield values from various laboratories gave variable results. In cooperation between Marv's laboratory and those of Frank Hinds and Fred Owens (Department of Animal Science), Ronald Isaacson set up model experiments with continuous cultures of mixed rumen bacteria growing on glucose to determine the efficiency of rumen bacterial growth in relationship to the rate of passage of material through the system. They found that at dilution rates covering the range expected in the rumen, the bacterial protein yield varied as much as two-fold. This variation emphasized the importance of the bacterial maintenance energy requirement in net growth of rumen bacteria. As rates of passage increased, more of the energy of the digested material available to the ruminant animal was used for growth and synthesis of microbial protein. This concept was later exploited in many

laboratories to improve the efficiency of protein synthesis by rumen microbes.

Identity of the bacteria active in urea hydrolysis to ammonia and CO_2 via the production of urease in the rumen was long unknown. In the early 1980s Marv assigned Isaacson the special problem of selectively isolating major urease-forming bacteria, by use of urea as the main possible N source in a chemically defined medium. They theorized that the bacteria might not form urease in the presence of much ammonia or other rapidly used N source present in the growth medium. They successfully isolated a urease-producing bacterium that was shown by graduate student Andrew John to be a somewhat atypical variety of *Selenomonas ruminantium*. In this strain, urease was indeed strongly repressed by large amounts of urea, ammonia, or amino acids. Varel, using similar isolation techniques, showed that the human bowel organism, *Peptostreptococcus productus*, present in huge numbers in human feces, had similar urease activity. Graduate student Mary Ann Wozny then developed a rapid assay and growth medium in which most pure cultures of fermentative anaerobes grew and expressed urease activity. In screening many nonselectively isolated strains from the rumen and human species, she confirmed Varel's results with feces and found more human fecal anaerobes forming urease as well as more rumen species. Such strains were found to be predominantly from cattle maintained on high grain diets with unusually low levels of crude protein and thus low rumen ammonia levels. The strains were sent by Marv to Virginia Polytechnic Institute and identified as *S. dextrinosolvens, Treponema* spp and *Ruminococcus bromii.* Further studies with graduate student C. J. Smith and colleague R. B. Hespell, Marv studied urease regulation and enzymology of ammonia assimilation *in S. ruminantium*. The first enzyme of ammonia

assimilation was found to be glutamate dehydrogenase, which required little energy for ammonia assimilation but which had poor affinity for ammonia and was much less active at low ammonia levels. An alternative route was the glutamine synthetase-glutamate synthase system, which had a very high affinity for ammonia but required some of the energy otherwise available for the growth of the bacterium. Both the urease and the glutamine synthetase were strongly repressed when the growth rate was limited by the amount of energy source in the medium rather than by the ammonia level. Marv and coworkers hypothesized that synthesis of both urease and glutamine synthetase was regulated by a common gene product.

There was considerable controversy concerning the concentration of ammonia necessary in the rumen to ensure maximum growth rate and yield of the fermentative rumen bacteria. Marv, along with graduate student Dan Schaefer and Professor Carl Davis, proved that important rumen bacterial species had a great affinity for ammonia as nitrogen source and could achieve maximum growth rates with 1 mM or less ammonia.

Marv's graduate student Bill Brulla, in association with Professor Smith, studied the improved feed efficiency in cattle fed the bacterial antibiotic monensin. Furthermore, the nutrition of gastrointestinal tract anaerobes remained of interest to Marv. Janice Herbeck and he showed that the nutrient requirements of *R. bromii,* one of the main species digesting starch in humans and in ruminants fed large amounts of grain, were very similar to those of the cellulolytic *R. albus.* Varel determined the simple nutritional requirements of *Bacteroides fragilis,* the most numerous species in humans, a major starch and hemicellulose fermenter, and an opportunistic pathogen in compromised humans. The minimal medium developed by Varel and Marv

has since been used in many ecological, genetic, and pathology research laboratories.

Marv had long suspected that some important rumen bacteria had a growth requirement for a fat-soluble vitamin of the vitamin K group. Undergraduate student Colleen O'Dowd and graduate student Jane Leedle established this for *Succinivibrio* and Rogelio Gomez-Alarcon of Marv's group found 1,4-naphthaquinone to be the most active.

Marv's undergraduate student H. G. Betian and graduate student Barbara Lineham found large numbers of cellulolytic bacteria in the bowel microbiota in some young adult humans and isolated a new species of *Bacteroides.* Their finding of populations as high as 10^8 cellulolytic bacteria per g of feces had never been observed before in the bowel.

In another collaboration with Professor Davis, Barbara Genthner found that a lactate fermenter, *Eubacterium limosum*, which had been found earlier by Marv to exist only in the rumen of very young calves, was a very dominant organism in the rumen of sheep fed sugar cane molasses as their main energy source; it was also found to be a significant component in anaerobic digestors of domestic sewage. They established that the organism produced acetate and butyrate and some fatty acids with longer chains when it fermented the one-carbon compound methanol (from pectin breakdown) and H_2-CO_2. It also fermented branched-chain amino acids to branched-chain fatty acids, which were shown to be the major microbial products in the rumen of molasses-fed animals.

Marv served his scientific community well. He was the editor in chief of the *Journal of Applied and Environmental Microbiology* from 1967 to 1980 and was a member of the Board of Trustees of *Bergey's Manual of Determinative Bacteriology* from 1975 to 1986. He was a member of the American Society for Microbiology and the American Dairy

Science Association and a fellow of the American Association for the Advancement of Science and the American Academy of Microbiology. He also was a member of Phi Beta Kappa, Phi Kappa Phi, and Sigma Xi honor societies. For his contributions to science he received the Superior Service Award of the U.S. Department of Agriculture in 1959; the Borden Award of the American Dairy Science Association in 1978; the Paul A. Funk Award of the University of Illinois in 1979; the Fisher Award of the American Society for Microbiology in 1986; election to the National Academy of Sciences in 1987; the Alumni Achievement Award of Washington State University in 1991; and the Bergey's Medal for Distinguished Achievement in Bacterial Taxonomy in 1996. He also was made honorary member of the American Society for Microbiology, the highest honor awarded by that society.

In reviewing his many years of work in rumen and related anaerobic bacteriologic research, Marv concluded as follows:

> I became increasingly aware of my good fortune in having selected an area of work in which I have some innate competence and in having been at the right places at the right times to be associated with many outstanding, creative, unselfish colleagues whose main goals in life have been to advance the science of anaerobic bacteria and related areas in a holistic, unparochial, objective manner. I have also been very fortunate to have worked with administrators who put up with my somewhat juvenile personality and left me in the position that the major deterrents to achievement have been my own inadequacies. My wife, Margaret, has been the strong pillar of love and support essential to my progress.

Marv was the gentle giant of rumen microbiology. There was a special light in his eyes and a special tone to his voice when his beloved rumen bacteria were being discussed. He was a national treasure of information on anaerobes and

his colleagues at Illinois and all over the world benefited from his presence.

Marv is survived by his wife, Margaret, of 54 years; sons Robert M. Bryant of Livermore, California, and Steven E. Bryant of Champaign, Illinois; daughters Margaret ("Peggy") Bryant of Pleasanton, California, Susan J. Bryant of Olympia, Washington, and Katherine B. Smith of Maple Plain, Minnesota; sister June Chambers of Boise, Idaho; and nine grandchildren.

MOST OF THE information in this article was derived from an autobiographical article written in 1980 by Marvin P. Bryant in honor of his receipt of the Paul A. Funk Award of the University of Illinois. He titled it "Marvin P. Bryant, A Rumen Microbiologist."

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